E11 cont.

47. (New) A method according to claim 7, 8, 9 or 25, wherein said plurality of locations are provided on a semi-solid support.

48. (New) A method according to claim 47, wherein said semi-solid support is a gel.

REMARKS

Claims 1, 2, 4-17, 21 and 23 were pending in the application. In the instant Amendment, claim 23 has been canceled without prejudice, claims 1, 2, 4, 7-17 and 21 have been amended, and new claims 25-48 have been added to more clearly claim the present invention. Upon entry of the above-made amendments, claims 1, 2, 4-17, 21 and 25-48 will be pending in the present application. A marked version showing changes made to the amended claims is attached hereto as Exhibit A. A clean version of the pending claims, as amended, is attached hereto as Exhibit B.

Claims 1 has been amended to recite that in step c) a given nucleotide in labeled and unlabeled form is provided and that in step e) steps c) and d) are repeated for at least 19 times. Support for the amendment is found in the specification at page 16, lines 15-22 and at page 37, lines 10-12. Claim 1 has also been amended to make the language clearer.

Claim 7 has been amended to be in independent form to more clearly recite a method that comprises providing 10 or more locations with single stranded nucleic acid molecules and carrying out the sequencing steps on the nucleic acid molecules at the 10 or more locations. Claim 7 has also been amended to recite that in step b) a given nucleotide in labeled and unlabeled form is provided and that the ratio of said labeled and unlabeled form is chosen such that labeled nucleotides are used in primer extension less than 50% of the time. Support for the amendment is found in the specification at page 16, lines 15-22. Claims 8-9 have been amended to depend on claim 7. Claims 8-9 have also been amended to recite that 100 or more or 1000 or more nucleic acid molecules having different sequences are fully or partially sequenced at *100 or more* or *1000 or more* different locations, respectively (emphasis added). Support for the amendments is found in the specification at, e.g., page 12, line 22 through page 13, line 2.

Claim 12 has been amended to recite that one of the four nucleotides is dTTP or dUTP. Support for the amendment is found in the specification at page 16, lines 2-5.

Claim 15 has been amended to be in independent form to more clearly recite a method of sequencing by nick translation by reciting explicitly steps using nick translation. Claim 15 has also been amended to recite that in step c) a given nucleotide in labeled and unlabeled form is provided and that the ratio of said labeled and unlabeled form is chosen such that labeled nucleotides are used in nick translation less than 50% of the time. Support for the amendment is found in the specification at page 16, lines 15-22.

Claim 16 has been amended to be in independent form to more clearly recite the claimed method which involves providing at each of the first and second locations a single stranded nucleic acid molecule that is hybridized to a primer and sequencing said single single stranded nucleic acid molecule. Claim 16 has also been amended to recite that the method further comprises the following steps: f) removing said extended primers at said first and second locations; g) hybridizing the single stranded nucleic acid molecules at said first and second locations to a second primer such that said single nucleic acid molecules can serve as templates for extension of the 3' end of the primers; h) providing each said first and second location with a polymerase and one or more types of label-free nucleotide under conditions allowing template-directed extension of the primer to produce a primer extension product; i) providing each said first and second location with a nucleic acid polymerase and a given labelled nucleotide under conditions that allow template-directed extension of said primer extension product; j) detecting whether or not said labelled nucleotide has been used for extension of said primer extension product at each said first and second location by determining whether or not the label present on said nucleotide has been incorporated into said primer extension product, and if said labelled nucleotide has been used in extension of said primer extension product this step further comprises detecting how many of said nucleotides have been used; and k) repeating steps i) and j) one or more times so that extended primers each comprising a plurality of labels are provided and the sequences of the nucleic acid molecules at the first and second locations are obtained by reference to the number and type of nucleotides used in primer extension at said first and second locations. Support for the amendments is found in the specification at, e.g., page 18, line 18 through page 19, line 2.

Claim 17 has been amended to recite that the ratio of said labeled and unlabeled form is chosen such that labeled nucleotides are used in primer extension less than 50% of the

time. Support for the amendment is found in the specification at page 16, lines 15-22. Claim 17 has also been amended to delete the recitation of "without removing incorporated labels."

Claim 21 has been amended to recite that the method further comprises the following steps: (g) removing said extended primer; (h) hybridizing the target nucleic acid to a second primer to produce a second hybridized target nucleic acid/primer such that the target nucleic acid can serve as a template for extension of the 3' end of the primer; (i) incubating said second hybridized target nucleic acid/primer with a polymerase and one or more types of label-free nucleotide under conditions allowing template-directed extension of the primer to produce a second primer extension product; (j) incubating said second primer extension product with a polymerase and a type of nucleotide bearing a label under conditions allowing template-directed primer extension; (k) measuring the label incorporated into said second primer extension product to determine whether, and if so, by how many base increments, said second primer extension product has been extended by incorporation of the nucleotide type; (l) incubating said second primer extension product with a polymerase and a type of nucleotide bearing a label different from the label of step (j) under conditions allowing template-directed extension of the primer; (m) measuring incremental label incorporated into said second primer extension product in step (l) to determine whether, and if so, by how many base increments, said second primer extension product has been extended by incorporation of the nucleotide type of step (l); and (n) repeating steps (j) - (m) so that extended second primer comprising a plurality of labels is provided, until a desired portion of the target sequence can be determined from the incremental base incorporations into the second primer. Support for the amendments is found in the specification at, e.g., page 18, line 18 through page 19, line 2. Claim 21 has also been amended to delete the recitation of "without removing incorporated labels." Claim 21 has also been amended to make the language clearer.

Claims 2 and 10-14 have been amended to make the language clearer.

New claims 25-48 have been added. Support for the new claims is found in the specification at page 8, lines 21-24; page 10, lines 14-15; page 11, line 8-12; page 12, line 25 through page 13, line 1; page 13, lines 4-7; page 14, lines 9-17; page 16, lines 2-5; page 16, lines 15-22; page 18, line 5 through page 19, line 2; and page 22, lines 8-12.

No new matter has been added. Entry of the foregoing amendments and consideration of the following remarks are respectfully requested.

THE REJECTIONS UNDER 35 U.S.C. § 112, SECOND PARAGRAPH SHOULD BE WITHDRAWN

Claims 1, 2, 4-17, 21 and 23 are rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claim 1, 2 and 4-17 are rejected for the recitation of "such bases." The Examiner contends that the phrase is vague and does not specify the identity of the base. Applicants have amended claims 1 and 17 such that the phrase has been removed as unnecessary since the skilled artisan would understand that extension in such a situation would occur only if the complementary base were present at the appropriate position. The rejection is therefore obviated and should be withdrawn.

Claims 1, 2 and 4-17 are also rejected for the recitations of "each location" and "at these locations." The Examiner contends these phrases are unclear since they do not specify a particular location. Applicants have amended the claims to explicitly recite the first and second locations. The rejection is therefore obviated and should be withdrawn.

Claim 2 is rejected for the recitation of "converting all or part of the sequence obtained in step e) to its complementary sequence." The Examiner contends that it is not clear as to what is intended to be meant by "converting." Applicants have amended the claim to make the language more clearer by reciting "determining the complementary sequence of all or part of the sequence obtained in step e)." The rejection is therefore obviated and should be withdrawn.

Claims 21 and 23 are rejected for the recitation of "the hybridized target nucleic acid/primer." The Examiner contends that the phrase lacks proper antecedent basis. Applicants have canceled claim 23 and amended claim 21 to recite in step (a) production of a hybridized target nucleic acid/primer. The rejection is therefore obviated and should be withdrawn.

Claim 21 and 23 are also rejected for the recitation of "the incremental base additions." The Examiner contends that the phrase lacks proper antecedent basis. Applicants have canceled claim 23 and amended claim 21 to recite "the incremental base incorporations." The rejection is therefore obviated and should be withdrawn.

THE REJECTION UNDER 35 U.S.C. § 102(e)

SHOULD BE WITHDRAWN

Claims 1, 2, 4-15, 21 and 22 are rejected under 35 U.S.C. § 102 (e) as being anticipated by Rosenthal et al., U.S. Patent No. 6,087,095 ("Rosenthal"). Applicants respectfully disagree with the Examiner's rejection for reasons set forth below.

A claim is anticipated under 35 U.S.C. § 102 only if each and every element and limitation as set forth in the claim is found, either expressly described or inherently present, in a single prior art reference. *Glaxo, Inc. v. Novopharm Ltd.*, 52. F.3d 1043, 1047 (Fed. Cir. 1995). There must be *no differences* between the claimed invention and the reference disclosure as viewed by a person of ordinary skill in the field of the invention. *Scripps Clinic & Research Fdn. v. Genentech, Inc.* 927 F. 2d. 1565, 1576 (Fed. Cir. 1991).

At the outset, Applicants respectfully submit that claim 22 was not pending in the application. The rejection of claim 22 is therefore in error and should be withdrawn. Rosenthal teaches a method of DNA sequencing comprising sequentially adding nucleotides to a primer annealed to a DNA template. Rosenthal does not teach a method in which *at least 19 cycles* of sequencing are performed without removing incorporated labels, as recited in the amended claim 1 and claims dependent thereon (emphasis added). Rosenthal does not teach a method in which *a given nucleotide in labeled and unlabeled form is provided, wherein the ratio of the labeled and unlabeled form is chosen such that labeled nucleotides are used in primer extension less than 50% of the time*, as recited in the amended claim 7 and 15 and claims dependent thereon (emphasis added). Nor does Rosenthal teach a method of sequencing a single nucleic acid molecule annealed with a primer, comprising extending the primer with labeled nucleotides and detecting incorporations of labeled nucleotides, removing the primer extension product, reannealing with a second primer, extending the second primer with label-free nucleotides, and extending the primer further with labeled nucleotides and detecting incorporations of labeled nucleotides, as recited in the amended claims 16 and 21. Thus, Applicants submit that Rosenthal does not anticipate the claimed methods, as amended, of the present invention. Accordingly, Applicants respectfully request that the rejections under 35 U.S.C. § 102 (e) be withdrawn.

THE REJECTIONS UNDER 35 U.S.C. § 103(a)
SHOULD BE WITHDRAWN

Claims 15 and 23 are rejected under 35 U.S.C. § 103 (a) as being unpatentable over Rosenthal et al., U.S. Patent No. 6,087,095 ("Rosenthal") in view of Fu et al., Nucleic Acid Res. 25:677-679 ("Fu"); and claim 16 is rejected under 35 U.S.C. § 103 (a) as being unpatentable over Rosenthal in view of Rabani, WO 96/27025 ("Rabani"). Applicants respectfully disagree with the Examiner's rejections for reasons set forth below.

A finding of obviousness under 35 U.S.C. § 103(a) requires a determination that the differences between the claimed subject matter and the prior art are such that the subject matter as a whole would have been obvious to one of ordinary skill in the art at the time the invention was made. *Graham v. Deere*, 383, U.S. 1 (1956). The relevant inquiry is whether the prior art suggests the invention and whether the prior art provides one of ordinary skill in the art with a reasonable expectation of success. Both the suggestion and the reasonable expectation of success must be found in the prior art. *In re Vaeck*, 947 F.2d 488 (Fed. Cir. 1991).

At the outset, Applicants respectfully point out that claim 23 has been canceled. The rejection of claim 23 under 35 U.S.C. § 103(a) is therefore obviated and should be withdrawn. With respect to claim 15, as discussed above, Rosenthal does not teach a method in which Rosenthal does not teach a method in which *a given nucleotide in labeled and unlabeled form is provided, wherein the ratio of the labeled and unlabeled form is chosen such that labeled nucleotides are used in primer extension less than 50% of the time* (emphasis added). Fu teaches a method for sequencing of dsDNA by strand displacement or nick translation. In Fu, "duplex probes with five base single-stranded overhangs can capture dsDNA targets .... Ligation generates a predesigned nick site, where DNA polymerase can *generate sequencing ladders* by strand displacement or nick translation ...." (Fu, Abstract; emphasis added) The sequencing ladders generated are then sequenced by using a DNA sequencer. Therefore, Fu teaches generation of sequencing ladders by strand displacement or nick translation and subsequent sequence determination by gel electrophoresis of such sequencing ladders. Fu does not teach or suggest a method in which a given nucleotide in labeled and unlabeled form is provided, wherein the ratio of the labeled and unlabeled form is chosen such that labeled nucleotides are used in primer extension less than 50% of the time. Therefore, Fu does not add what is missing in Rosenthal. Rosenthal and Fu provide no suggestion with a reasonable expectation of success of a method of DNA sequencing in

which a given nucleotide in labeled and unlabeled form is provided, wherein the ratio of the labeled and unlabeled form is chosen such that labeled nucleotides are used in primer extension less than 50% of the time. Therefore, claim 15 is not obvious over Rosenthal in view of Fu, and the rejection of claim 15 under 35 U.S.C. § 103(a) over Rosenthal in view of Fu should be withdrawn.

With respect to the rejection of claim 16 over Rosenthal in view of Rabani, as discussed above, Rosenthal does not teach or suggest a method of sequencing comprising extending primer with labeled nucleotides and detecting incorporation(s) of labeled nucleotides, removing the primer extension product, reannealing with a second primer, extending the second primer with label-free nucleotides, and extending the primer further with labeled nucleotides and detecting incorporation(s) of labeled nucleotides. Rabani teaches methods for sequencing single polynucleotide molecules. However, Rabani does not teach or suggest extending primer annealed to a template with labeled nucleotides and detecting incorporation(s) of labeled nucleotides, removing the primer extension product, reannealing with a second primer, extending the second primer with label-free nucleotides, and extending the primer further with labeled nucleotides and detecting incorporation(s) of labeled nucleotides. Thus Rabani does not add what is missing in Rosenthal. Therefore, claim 16 as amended is not obvious over Rosenthal in view of Rabani, and the rejection of claim 16 under 35 U.S.C. § 103(a) over Rosenthal in view of Rabani should be withdrawn.

CONCLUSION

Applicants respectfully request entry of the foregoing amendments and remarks into the file of the above-identified application. Applicants believe that each ground for rejection or objection has been successfully overcome or obviated, and that all the pending claims are

in condition for allowance. Withdrawal of the Examiner's rejections and allowance of the application are respectfully requested.

Respectfully submitted,

Date July 24, 2002

43,180

Gary Tanigawa (Reg. No.)

Nixon & Vanderhye, PC
1100 North Glebe Road
8th Floor
Arlington VA 22201-4714
703-816-4000

EXHIBIT A: MARKED VERSION OF THE AMENDED CLAIMS
U.S. APPLICATION SERIAL NO. 09/402,260
(ATTORNEY DOCKET NO. 1430-268)

(as amended July 24, 2002)

1. (Four times amended) A method for sequencing nucleic acid molecules, comprising the steps of:

a) providing at a first location a first plurality of single stranded nucleic acid molecules that have the same sequence as one another and that are hybridized to primers in a manner to allow template-directed primer extension when in the presence of nucleotides and a nucleic acid polymerase;

b) providing at a second location, which is different from the first location, a second plurality of single stranded nucleic acid molecules that have the same sequence as one another, but that have different sequence from the sequence of the single stranded nucleic acid molecules at the first location, and that are also hybridized to primers in a manner to allow template-directed primer extension when in the presence of nucleotides and a nucleic acid polymerase;

c) providing each said first and second location with a nucleic acid polymerase and a given [labelled] nucleotide in labeled and unlabeled form under conditions that allow template-directed extension of the primers [if a complementary base or if a plurality of such bases is present at the appropriate position in the single stranded nucleic acid molecules];

d) detecting whether or not said labelled nucleotide has been used for primer extension at each said first and second location by determining whether or not the label present on said nucleotide has been incorporated into extended primers, and if said labelled nucleotide has been used in primer extension this step [involves] further comprises detecting how many of said nucleotides have been used per extended primer[,]; and

e) repeating steps c) and d) [one or more] at least 19 times without removing incorporated labels so that extended primers each comprising a plurality of labels are provided;

whereby the [sequence] sequences of the nucleic acid molecules at the first and second locations [is] are obtained by reference to the number and type of nucleotides used in primer extension at [these location] said first and second locations.

2. (Twice Amended) A method according to claim 1, further comprising [converting] determining the complementary sequences of all or part of the [sequence] sequences obtained in step e) [to its complementary sequence].

4. (Twice Amended) A method according to claim 1, [wherein] further comprising after step c) removing excess nucleotides that have not been used in primer extension [are removed].

7. (Twice amended) A method [according to claim 1] for sequencing nucleic acid molecules, comprising the steps of:

a) providing at each of a plurality of locations a plurality of single stranded nucleic acid molecules that have the same sequence as one another but have different sequences from the sequence of the single stranded nucleic acid molecules at any other location among said plurality of locations, wherein said single stranded nucleic acid molecules are hybridized to primers in a manner to allow template-directed primer extension when in the presence of nucleotides and a nucleic acid polymerase;

b) providing each of said plurality of locations with a nucleic acid polymerase and a given nucleotide in labeled and unlabeled form under conditions that allow template-directed extension of the primers, wherein the ratio of said labeled and unlabeled form is chosen such that labeled nucleotides are used in primer extension less than 50% of the time;

c) detecting whether or not said labelled nucleotide has been used for primer extension at each of said plurality of locations by determining whether or not the label present on said nucleotide has been incorporated into extended primers and if said labelled nucleotide has been used in primer extension this

step further comprises detecting how many of said nucleotides have been used per extended primer; and

d) repeating steps b) and c) one or more times so that extended primers each comprising a plurality of labels are provided;

wherein the sequence of the nucleic acid molecules at each of said plurality of locations is obtained by reference to the number and type of nucleotides used in primer extension at each of said plurality of additional locations, and wherein said plurality of locations consists of 10 or more locations such that 10 or more nucleic acid molecules having different sequences are fully or partially sequenced at 10 or more different locations simultaneously.

8. (Three Times Amended) A method according to claim [1] 7, wherein said plurality of locations consists of 100 or more locations such that 100 or more nucleic acid molecules having different sequences are fully or partially sequenced at [10] 100 or more different locations simultaneously.

9. (Three Times Amended) A method according to claim [1] 8, wherein said plurality of locations consists of 1000 or more locations such that 1000 or more nucleic acid molecules having different sequences are fully or partially sequenced at [10] 1000 or more different locations simultaneously.

10. (Twice Amended) A method according to claim 1, wherein each of four different nucleotides is [used] provided in separate primer [extension] extensions.

11. (Amended) A method according to claim 10, wherein said four different nucleotides are [used] provided in a predetermined order in repeated cycles.

12. (Twice Amended) A method according to claim 10, wherein the four different nucleotides are (i) dATP, (ii) dTTP or dUTP, (iii) dGTP and (iv) dCTP in labelled [form] and unlabeled forms.

13. (Amended) A method according to claim 10, wherein the four different nucleotides are ATP, UTP, GTP and GTP in labelled [form] and unlabeled forms.

14. (Twice Amended) A method according to claim 1, wherein [the detection] step d) is carried out without removing the nucleic acid molecules from the [different] first and second locations.

15. (Three Times Amended) A method [as described in claim 1 with the exception that double stranded nucleic acid molecules having nicks therein are provided at the first and/or second locations instead of providing single stranded molecules hybridized to primers] for sequencing nucleic acid molecules, comprising the steps of:

a) providing at a first location a first plurality of double stranded nucleic acid molecules that have the same sequence as one another and that have nicks therein in a manner to allow nick translation when in the presence of nucleotides and a nucleic acid polymerase;

b) providing at a second location, which is different from the first location, a second plurality of double stranded nucleic acid molecules that have the same sequence as one another, but that have a different sequence from the sequence of the single stranded nucleic acid molecules at the first location, and that have nicks therein in a manner to allow nick translation when in the presence of nucleotides and a nucleic acid polymerase;

c) providing each said first and second location with a nucleic acid polymerase and a given nucleotide in labeled and unlabeled form under conditions that allow nick translation, wherein the ratio of said labeled and unlabeled form is chosen such that labeled nucleotides are used in nick translation less than 50% of the time;

d) detecting whether or not said labelled nucleotide has been used for nick translation at each said first and second location by determining whether or not the label present on said nucleotide has been incorporated into said double stranded nucleic acid molecules, and if said labeled nucleotide has been used

in nick translation this step further comprises detecting how many of said nucleotides have been used per translated nick; and

e) repeating steps c) and d) one or more times so that nick translation products each comprising a plurality of labels are provided;

whereby the sequences of the nucleic acid molecules at the first and second locations are obtained by reference to the number and type of nucleotides used in nick translation at each of said first and second locations.

16. (Twice Amended) A method [as described in claim 1 with the exception that only one nucleic acid molecule is provided at the first and/or second locations] for sequencing nucleic acid molecules, comprising the steps of:

a) providing at a first location a single stranded nucleic acid molecule that is hybridized to a primer in a manner to allow template-directed primer extension when in the presence of nucleotides and a nucleic acid polymerase;

b) providing at a second location, which is different from the first location, a single stranded nucleic acid molecule that has a different sequence from the sequence of the single stranded nucleic acid molecule at the first location, and that is also hybridized to a primer in a manner to allow template-directed primer extension when in the presence of nucleotides and a nucleic acid polymerase;

c) providing each said first and second location with a nucleic acid polymerase and a given labelled nucleotide under conditions that allow template-directed extension of the primers;

d) detecting whether or not said labelled nucleotide has been used for primer extension at each said first and second location by determining whether or not the label present on said nucleotide has been incorporated into extended primers, and if said labelled nucleotide has been used in primer extension this step further comprises detecting how many of said nucleotides have been used per extended primer; and

e) repeating steps c) and d) one or more times so that extended primers each comprising a plurality of labels are provided and the sequences of the nucleic acid molecules at the first and second locations are obtained by reference to the number and type of nucleotides used in primer extension at said first and second locations;

f) removing said extended primers at said first and second locations;

g) hybridizing the single standed nucleic acid molecules at said first and second locations to a second primer such that said single nucleic acid molecules can serve as templates for extension of the 3' end of the primers;

h) providing each said first and second location with a polymerase and one or more types of label-free nucleotide under conditions allowing template-directed extension of the primer to produce a primer extension product;

i) providing each said first and second location with a nucleic acid polymerase and a given labelled nucleotide under conditions that allow template-directed extension of said primer extension product;

j) detecting whether or not said labelled nucleotide has been used for extension of said primer extension product at each said first and second location by determining whether or not the label present on said nucleotide has been incorporated into said primer extension product, and if said labelled nucleotide has been used in extension of said primer extension product this step further comprises detecting how many of said nucleotides have been used; and

k) repeating steps i) and j) one or more times so that extended primers each comprising a plurality of labels are provided and the sequences of the nucleic acid molecules at the first and second locations are obtained by reference to the number and type of nucleotides used in primer extension at said first and second locations.

17. (Four Times Amended) A method for sequencing nucleic acid molecules, comprising the steps of:

a) providing at a first location a first plurality of single stranded nucleic acid molecules that have the same sequences as one another and that are hybridized

to primers in a manner to allow template-directed primer extension when in the presence of nucleotides and a nucleic acid polymerase;

b) providing at a second location, which is different from the first location, a second plurality of single stranded nucleic acid molecules that have the same sequences as one another, but that have different sequences from the sequences of the single stranded nucleic acid molecules at the first location, and that are also hybridized to primers in a manner to allow template-directed primer extension when in the presence of nucleotides and a nucleic acid polymerase;

c) providing each said first and second location with a nucleic acid polymerase and a given nucleotide in labelled and unlabelled form under conditions that allow template-directed extension of the primers [if a complementary base or if a plurality of such bases is present at the appropriate position in the single stranded nucleic acid molecules], wherein the ratio of said labeled and unlabeled form is chosen such that labeled nucleotides are used in primer extension less than 50% of the time;

d) detecting whether or not said labelled nucleotide has been used for primer extension at each location by determining whether or not the label present on said nucleotide has been incorporated into extended primers, and if said labelled nucleotide has been used in primer extension, this step [involves] further comprises detecting how many of said nucleotides have been used per extended primer;

e) repeating steps c) and d) one or more times [without removing incorporated labels] so that extended primers each comprising a plurality of labels are provided;

whereby the [sequence] sequences of the nucleic acid molecules at the first and second locations [is] are obtained by reference to the number and type of nucleotides used in primer extension at [these location] said first and second locations.

21. (Four Times Amended) A method of sequencing a target nucleic acid comprising:

(a) hybridizing the target nucleic acid to a primer to produce a hybridized target nucleic acid/primer whereby the target nucleic acid can serve as a template for extension of the 3' end of the primer,

(b) incubating the hybridized target nucleic acid/primer with a polymerase and a type of nucleotide bearing a label under conditions [supporting] allowing template-directed extension of the primer if the nucleotide type can be incorporated as the complement of a corresponding nucleotide of the target;

c) measuring first label incorporated into the primer to determine whether, and if so, by how [may] many base increments, the primer has been extended by incorporation of the nucleotide type;

(d) incubating the hybridized primer/target nucleic acid with a polymerase and a different type of nucleotide bearing a label under conditions [supporting] allowing template-directed extension of the primer if the different nucleotide type can be incorporated so as to be complementary to a corresponding nucleotide in the target:

(e) measuring incremental label incorporated into the primer due to the previous incubating step to determine whether, and if so, by how [may] many base increments, the primer has been extended by incorporation of the different nucleotide type; [and]

(f) repeating steps (b) - (e) [without removing incorporated labels] so that extended primer comprising a plurality of labels [are] is provided, until a desired portion of the target sequence can be determined from the incremental base [additions to] incorporations into the primer;

(g) removing said extended primer;

(h) hybridizing the target nucleic acid to a second primer to produce a second hybridized target nucleic acid/primer such that the target nucleic acid can serve as a template for extension of the 3' end of the primer;

(i) incubating said second hybridized target nucleic acid/primer with a polymerase and one or more types of label-free nucleotide under conditions

allowing template-directed extension of the primer to produce a second primer extension product;

(j) incubating said second primer extension product with a polymerase and a type of nucleotide bearing a label under conditions allowing template-directed primer extension;

(k) measuring the label incorporated into said second primer extension product to determine whether, and if so, by how many base increments, said second primer extension product has been extended by incorporation of the nucleotide type;

(l) incubating said second primer extension product with a polymerase and a type of nucleotide bearing a label different from the label of step (j) under conditions allowing template-directed extension of the primer;

(m) measuring incremental label incorporated into said second primer extension product in step (l) to determine whether, and if so, by how many base increments, said second primer extension product has been extended by incorporation of the nucleotide type of step (l); and

(n) repeating steps (j) - (m) so that extended second primer comprising a plurality of labels is provided, until a desired portion of the target sequence can be determined from the incremental base incorporations into the second primer.

Claim 23 has been canceled.

New claims 25-48 have been added.